ANTIHYPERTHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC PROPERTIES OF N-HEXANE FRACTION OF *HEINSIA CRINITA* CRUDE LEAF EXTRACTS

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ABSTRACT

Earlier studies had shown the n-hexane fraction of *Heinsia crinita* to contain phytochemicals known to have antihyperglycemic, hypocholesterolemic, hypolipidemic and antioxidant activities suggestive that its may be responsible, at least in part, for the antidiabetic action of the plant. The aim of this study was to establish if indeed the n-hexane fraction had antihyperglycemic, hypocholesterolemic and hypolipidemic properties. Thirty (30) albino Wistar rats of both sexes weighing 120-180g were randomly shared into five (5) groups of six animals each. Group 1 and 2 served as the normal control (NC) and diabetic control (DC) respectively and received placebo. Groups 3, 4 and 5 were diabetic treated groups and received 400mg/kg b.w Metformin, 400mg/kg b.w of crude extract (HC-C) and n-hexane fraction (HC-H) of *Heinsia crinita* respectively. Induction of diabetes resulted in elevated levels of blood glucose and progressive reduction of body weight. Administration of the n-hexane fraction, as with the crude extract and the standard drug Metformin, reversed the diabetes induced hyperglycemia and loss in body weight. Administration of the n-hexane fraction, as with the crude extract and standard drug Metformin, also significantly (p<0.05) lowered the elevated levels of TG, VLDL –C and LDL-C associated with diabetes (DC group) and increased the HDL-C level significantly (p<0.05) compared to DC. However the crude extract and fraction of *H. crinita* failed to lower the elevated TC level associated with diabetes. The n-hexane fraction, unlike the crude fraction did not lower the LDL-cholesterol level relative to DC. From the result, it can be
deduced that n-hexane fraction of *Heinsia crinita*, as with the crude fraction, has anti-hyperglycemic and to a large extent antihyperlipidemic potentials which can be exploited for management of diabetes and it related complications. Moreover, the n-hexane fraction will be more amenable to standardization than the crude extract.

**KEYWORDS:** *Heinsia crinita*; n-hexane fraction; anti-hyperglycemic; antihyperlipidemic; body weight.

**INTRODUCTION**

Diabetes mellitus, possibly the world’s largest growing metabolic disorder, is one of the major global public health problems. Recent survey estimated that there will be more than 439 million people suffering from diabetes in nearly all countries by the year 2030.[1] Diabetes mellitus is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism.[2,3] Persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS) which may ultimately result in destruction of some vital organs of the body.[4-6]

As the knowledge of the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases.[7] Current treatments have been proven to have significant side effects associated with them and fail to significantly alter the course of diabetic complications;[8] thus there is an increasing demand and search for antidiabetic agents from natural sources with fewer side effects. Candidates for this natural resource are the medicinal plants. Apart from their minimal side effects, there are cost effective, readily available and more acceptable by the rural populace. Ethno-botanical information reveals about 800 plants that may possess antidiabetic potential.[9] While some plant products act by lowering the level of glucose in the blood, others act by inhibiting glucose absorption from the gut and hence prevent the surge in blood glucose that can occur immediately after a meal.[10] Some of these plants have been validated in our laboratory.[3,11-15]

However, as was pointed out in our previous publication[16,17] the issue of standardization (among other Quality issues) of medicinal plant formulations remains a drawback as most of plant medicines are in the form of crude extracts which are a mixture of several ingredients which may vary in composition even in same species. For “true” standardization, constituents
with known therapeutic or pharmacological activity must be ascertained so as to use their concentration levels as a basis for standardization.

*Heinsia crinita* (also known as “Bush apple” and called “Atama” in our local Efik language) a persistent scrambling shrub with very conspicuous leafy calyx-lobes belonging to the family *Rubiaceae* and found across the tropical region from Guinea to Western Cameroon and Fernando Po, and across the Congo basin to East and South Central Africa\(^{18-20}\) has been shown in our laboratory to have hypoglycemic, hepatoprotective and nephroprotective effects in alloxan-induced Type 1 diabetes using albino Wistar rats.\(^ {21}\) As a prelude to identifying its therapeutic components, towards standardization of the plant, the crude extract of the plant was fractionated using n-hexane and the phytochemical constituents determined.\(^ {17}\) The components, among other effects, have been known to have antihyperglycemic, hypocholesterolemic, hypolipidemic and antioxidant activities suggestive that, like the crude plant extract, it may have antidiabetic activity.

This work aims at ascertaining if indeed the n-hexane fraction of *Heinsia crinita* has these suggestive antidiabetic related activities.

**MATERIALS AND METHOD**

**Preparation of leaf extract:** The plants material (leaves) where purchased from a local market in Akpabuyo Local Government Area of Cross River State, Nigeria. The leaves where authenticated in the Department of Botany, University Of Calabar, Nigeria as earlier published (Ebong *et al*., 2014). The collected leave material where thoroughly washed with tap water, rinsed with distilled water, allowed to drain then air dried for 7 days after which the leaves where grounded into powdered form with a manual blender. The powdered leaves where weighed using an electronic weighing balance and soaked in a 96% ethanol solution in the ratio of 1:4 for 48 hours with intermittent agitation. The mixture was doubly filtered using a cheese cloth and Whatman filter paper. The filtrate was then concentrated using a rotary evaporator and residual solvent removed by placing in a water bath at a temperature of 40-45°C.

**Fractionation of plant extract using column chromatography:** A standard column with a length of 60cm and diameter of 4.5cm was used for the chromatographic process. Silica gel of mesh 60-120 was suspended in distilled water to form a slurry and then loaded unto the column. The silica gel column was thereafter equilibrated with n-hexane. The crude extract
(30g) was reconstituted in n-hexane, loaded unto the column and eluted using 500ml of n-hexane. The eluted fractions were pooled and concentrated as for the crude extract.

**Experimental animals and treatment Protocol:** Thirty (30) albino Wistar rats of both sexes weighing between 120-180g were used for this study. These animals were randomly divided into five (5) groups of six rats each and treated according to the schedule in Table 1. The doses used where based on the established LD$_{50}$ from preliminary studies. The extracts where reconstituted in normal saline (vehicle) and administered via gastric intubation, twice a day, at twelve hourly interval. Treatment lasted for 14 days.

**Table 1: Animal distribution and treatment schedule**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No of animals</th>
<th>Treatment dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>6</td>
<td>Placebo (0.2％DMSO)</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control</td>
<td>6</td>
<td>Placebo (0.2％DMSO)</td>
</tr>
<tr>
<td>3</td>
<td>Metformin</td>
<td>6</td>
<td>Metformin (500mg/70kg b.w)</td>
</tr>
<tr>
<td>4</td>
<td>Crude extract</td>
<td>6</td>
<td>Crude extract (400mg/kg b.w)</td>
</tr>
<tr>
<td>5</td>
<td>n-hexane fraction</td>
<td>6</td>
<td>N-hexane (400mg/kg b.w)</td>
</tr>
</tbody>
</table>

**Experimental induction of diabetes:** Diabetes was induced in the experimental animals by intraperitoneal injection of 100mg/kg bw of alloxan monohydrate (Sigma–Aldrich Inc, St. Louis, Mo, USA) in sterile saline after a 12hr fast. After five (5) days of alloxan injection, diabetes was confirmed in the rats if fasting blood glucose (FBG) as determined using a glucometer (Acon laboratories Inc., San diego U.S.A) on blood obtained from the tail vein of the rats, was above 180mg/dl.

**Measurement of glucose levels and changes in body weight:** Glucose levels and changes in body weight were measured every three (3) days with the use of a glucometer and an electronic weighing balance respectively.

**Collection of blood samples for Lipid analysis:** At the end of 14 days, the animals were sacrificed twelve hour after the last drug administration and after an overnight fast. Whole blood was collected via cardiac puncture using sterile syringe and needle. Sera was obtained for lipid analysis by allowing the blood to stand for two (2) hours at room temperature to clot before centrifugation at 3,000 rpm for 10minutes using a bench top centrifuge (MSE, England) to separate cells from serum. Sera obtained were carefully removed using Pasteur pipettes and put into dry plastic specimen bottles. These were kept frozen in a refrigerator until when needed for lipid analysis.
Estimation of lipid parameters
Total Serum cholesterol (TC), High Density Lipoprotein-Cholesterol (HDL-C) and Triglycerides (TG) where estimated using Randox test kits according to the manufacturers protocol. LDL Cholesterol (LDL-C) concentration was calculated by subtraction of the sum of HDL cholesterol+ triglyceride from the total cholesterol concentration (TC) according to Friedewald et al.\textsuperscript{[22]} VLDL-Cholesterol concentration were taken as one fifth of the triglyceride concentrations.

Statistical analysis
Data was presented as Mean ± Standard error of mean. Data was computed and analysed using one way ANOVA and unpaired Student’s t-test with the help of a statistical package, SPSS version 18.0 for Windows. P values <0.05 were considered to be significant.

RESULT
Blood glucose changes
The blood glucose changes for the various treatment groups is presented in Figure 1. There was a significant increase in blood glucose level in the diabetic control group when compared to the normal control. Treatment of the animals with plant extracts HC-C and HC-H brought about a significant reduction in the glucose levels when compared to the diabetic control. The reductions in glucose levels by the HC-C and HC-H group had the same trend as the metformin treated group with both groups even outperforming that of the metformin standard drug.

![Blood glucose changes in the various experimental groups. Values are expressed as mean ± SEM, n = 6.](image-url)
Body weight changes: The body weight changes of the various experimental groups is presented in figure 2. In the course of the experiment, there was a progressive decrease in body weight of the diabetic control animals when compared to the normal control group. Treatment with the plant extracts HC-C and fraction HC-H brought about a progressive increase in body weight. The weight gain in the n-hexane fraction treated group though lower than that of the crude extract and NC was comparable to that of the standard drug.

![Graph showing body weight changes in various experimental groups]

**Fig 2:** Body weight changes in the various experimental groups. Values are expressed as mean ± SEM, n = 6.

Evaluation of the serum lipid parameters: Total serum cholesterol (TC), Total triglycerides (TG), low density lipoproteins cholesterol (LDL-C), very low density lipoproteins cholesterol (VLDL-C) and high density lipoproteins cholesterol (HDL-C) of the experimental animal groups, 14 days after treatment, is shown in Figure 3. The concentrations of total serum cholesterol (TC), Total triglycerides (TG), low density lipoproteins cholesterol (LDL-C) and very low density lipoproteins cholesterol (VLDL-C) of the diabetic control (DC) were significantly increased when compared to the normal control (NC) (P<0.05). The concentration of the high density lipoproteins cholesterol (HDL-C) in DC was however decreased relative to NC (P<0.05). All Treatment reversed the elevated diabetes-induced increased levels of TG and VLDL. Only the standard drug metformin reversed the diabetes induced TC increases. Treatment with HC-C and Metformin but not HC-H decreased the elevated level of LDL-C in DC towards NC levels. All treatments reversed the depressed levels of HDL-C in DC towards the NC levels.
Fig 3: Serum Lipid concentrations of the different experimental groups. Values are expressed as mean ± SEM, n = 6.

DISCUSSIONS
A wide array of plant derived active principles, representing numerous chemical compounds, have demonstrated activity consistent with their possible use in the treatment of diabetes.\[10,23\] Among them are alkaloids, glycosides, galactomannan gum, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions.

Previous studies in our laboratories has shown *Heinsia crinita* to have hypoglycemic, hepatoprotective and nephroprotective effects suggestive of its possible use in the management of diabetes.\[21\] A drawback to the use of medicinal plants in the management of diseases generally is the difficulty in standardizing the medicinal plant preparations. As a prelude to possible standardization we have started a systematic fractionation of the plant extract to localize the antidiabetic activity of the plant and determine the phytochemical constituents of such fraction(s). The n-hexane fraction in our previous study had been shown to contain active principles known for, among others, antihyperglycemic, hypocholesterolemic, hypolipidemic and antioxidative activities.\[17\]

In this study, all the groups induced with diabetes using alloxan showed very high serum glucose level. This is consistent with other studies.\[21,24,25\] This hyperglycemic condition may be due to pancreatic β-cells necrosis mediated by alloxan or simply due to the decrease in serum insulin or as a result of both processes.\[21\] HC-H from our previous study\[17\] was shown to have a preponderance of Phytol a precursor for the manufacture of synthetic forms of vitamin E\[26\] and Vitamin K1\[27\] which serves as antioxidant. Oleic, n-hexadecanoic and
11-Octadecenoic acid, other key components of HC-H[17] have also been known to have antioxidant activities.[28] 11-Octadecenoic acid has also been reported to protect cells from oxidant-induced injury.[29] The significant decreases in blood glucose levels in HC-H, and by extension HC-C, may have been as a result of the regeneration of the Islets of Langeham (resulting in normal insulin levels) occasioning the mopping up of the cell destructive free radicals by these antioxidant phytochemicals present in the n-hexane fraction. Besides, Phytanic acid (PA), a chorophyll metabolite from phytol, has been shown to have potentials in regulating glucose metabolism by regulating hepatic glucose homeostasis.[30] Also, oleic acid has been shown to increase insulin production by pancreatic cells.[31] Heptacosanoic acid, another key component of HC-H[17] has been reported to exhibit inhibitory activity against $\alpha$-glucosidase in a dose-dependent manner[32] and may be contributory to the observed anti-hyperglycaemic effect of HC-H.

The body weight of the rats taken at three days interval after induction of diabetes showed a significant decrease in mean body weight of the rats in the untreated diabetic group. This observation is in consonance with other works.[21,33] Studies have shown an association between hypoglycemia and decreased body weight in animal models including man.[11,34] Tissue wasting is characteristic of poor glycemic control in diabetes mellitus and this usually enhances protein and fat mobilization, resulting in weight loss.[35] Diabetic rats in groups 4 and 5 treated with HC –C and HC – H showed a steady increase in mean body weight. This compared favourably with the metformin – treated group. This showed that Heinsia crinita n-hexane fraction, as was the crude extract, was able to protect the albino Wistar rats from tissue wasting associated with diabetes mellitus and hence weight loss.

Hyperlipidemia is one of the clinical features of alloxan – induced type 1 diabetes mellitus.[12,25,33,36-39] In this study, alloxan induced elevated levels of TC, TG, LDL-C, VLDL-C and decreased level of HDL-C in rats in diabetic control group. Abnormalities in glucose and fatty acid metabolism due to altered insulin action in fat cells results in dyslipidemia[40] as do excess mobilization of fats from adipose tissues.[41] This alteration, in combination with disturbance of cell membrane integrity of adipose tissues (membrane lipid peroxidation), may be among the probable factors causing raised total serum cholesterol and triglyceride levels on exposure to alloxan and its metabolites.

The results from this study showed that n-Hexane fraction of Heinsia crinita, like the crude extract, lowered TG and VLDL-C and raised HDL-C levels in diabetic rats when compared
to diabetic control rats. The n-Hexane fraction of *Heinsia crinita* as earlier pointed out is rich in phytol and several unsaturated fatty acids. The hypolipidemic effect of HC-H may be attributed to the action of phytanic acid, a metabolite of phytol. Phytanic acid induces activation of PPARs/RXR heterodimerization; this dimer induces various genes involved in lipid homeostasis\[^{42}\] and may be contributory to the hypolipidemic effect of this fraction. Another study suggested that feeding 11-Octadecenoic acid, a component of HC-H, to rats lowered total cholesterol, lowered LDL cholesterol and lowered triglyceride levels.\[^{43}\] HC-H, like HC-C, failed to lower the diabetes induced increases in TC in spite of the presence of Oleic, n-hexa-decanoic and 11-Octadecenoic acid\[^{17}\] which have been reported to play a key role in the reduction of cholesterol in blood (hypcholesterolemic effect).\[^{28}\] The inability of HC-H and HC-C to lower TC in this study may be due to the duration of the treatment. The high LDL cholesterol levels in the diabetic control though reversed by the plant crude extract was not reversed by the n-hexane fraction. It is probable that some active principle present in the crude plant extract, that is responsible for lowering LDL, is absent in the n-hexane fraction.

In summary, the n-hexane fraction of *Heinsia crinita*, as suggestive by its phytochemical composition from our earlier work\[^{17}\] had indeed antiglycemic and to a large extent anti-hyperlipidemic effect. Despite the presence of phytochemical components with hypocholesterolemic effect, hypocholesterolemia was not demonstrated in the plant crude extract and fraction ostensibly because of the short duration of treatment. This will be an issue for further studies.

REFERENCES


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